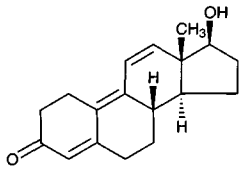
Trenbolone



Molecular formula: $C_{18}H_{22}O_2$
Molecular weight: 270.37
CAS Registry No.: 10161-33-8, 10161-34-9 (acetate)
Merck Index: 9716

SAMPLE

Matrix: bile, feces

Sample preparation: Condition a Bond-Elut CN SPE cartridge with 4 mL chloroform and 5 mL petroleum ether. Feces. 10 g Feces + 100 μL β-glucuronidase (type H2 Helix pomatia, Sigma) + 25 mL 200 mM pH 5.5 sodium phosphate buffer, let stand overnight at room temperature or heat at 37° for 4 h, add 60 mL MeOH, shake for 1 h, centrifuge at 4° at 2000 g for 10 min. Remove 50 mL of the supernatant and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 10 mL buffer, add 10 mL MTBE, shake vigorously for 1 min, centrifuge at 4° at 2000 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 3 mL diethyl ether, sonicate, add 3 mL petroleum ether (bp 40-60°, add to the SPE cartridge, rinse flask with 1 mL aliquots of petroleum ether:diethyl ether 50:50, add the rinses to the SPE cartridge, wash the SPE cartridge with 3 mL petroleum ether, wash with 4 mL petroleum ether:chloroform 50:50, elute with 4 mL chloroform, evaporate he eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL MeOH, sonicate, add 2 mL isotonic PBS, vortex. Apply the mixture to an immunoaffinity column (preparation details in paper), wash with 3 mL water, elute with 3 mL EtOH:water 70:30. Evaporate the eluate to dryness under a stream of nitrogen at 70°, reconstitute the residue in 100 μL MeCN, sonicate for 10 min, add 100 μL water, vortex, inject a 50 μL aliquot. Bile. 5 mL Bile + 50 μL β-glucuronidase (type H2 Helix pomatia, Sigma) + 12.5 mL 200 mM pH 7.0 sodium phosphate buffer, let stand overnight at room temperature or heat at 37° for 4 h, add to an Extrelut 20 column, let stand for 20 min, elute with two 20 mL portions of diethyl ether (let column run dry between each addition of ether), evaporate to dryness under reduced pressure at 30°, reconstitute the residue in 3 mL diethyl ether, sonicate, add 3 mL petroleum ether (bp 40-60°, add to the SPE cartridge, rinse flask with 1 mL aliquots of petroleum ether:diethyl ether 50:50, add the rinses to the SPE cartridge, wash the SPE cartridge with 3 mL petroleum ether, wash with 4 mL petroleum ether:chloroform 50:50, elute with 4 mL chloroform, evaporate he eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL MeOH, sonicate, add 2 mL isotonic PBS, vortex. Apply the mixture to an immunoaffinity column (preparation details in paper), wash with 3 mL water, elute with 3 mL EtOH:water 70:30. Evaporate the eluate to dryness under a stream of nitrogen at 70°, reconstitute the residue in 100 μL MeCN, sonicate for 10 min, add 100 μL water, vortex, inject a 50 μL aliquot. (Buffer was 100 mM pH 12.5 glycine-NaOH containing 100 mM NaCl.)

HPLC VARIABLES

Column: 125 × 4 LiChrosorb RP-18 endcapped
Mobile phase: MeCN:100 mM ammonium acetate 45:55
Flow rate: 1
Injection volume: 50
Detector: MS, Vestec Model 201A thermospray, electron beam 250 μA, electron multiplier 1600 V, source block 260°, tip heater 260°, lens assembly 135°, vaporizer probe 190°, m/z 271, SIM

CHROMATOGRAM

Retention time: 4 (17α-trenbolone)
Limit of detection: 0.5 ng/g (feces), 0.5 ng/mL (bile)

KEY WORDS

cow; SPE

REFERENCE

Hewitt,S.A.; Blanchflower,W.J.; McCaughey,W.J.; Elliott,C.T.; Kennedy,D.G. Liquid chromatography-thermospray mass spectrometric assay for trenbolone in bovine bile and faeces, *J.Chromatogr.*, **1993**, *639*, 185–191.

SAMPLE

Matrix: bile, tissue, urine

Sample preparation: Urine. 50 mL Urine + 6 g Amberlite XAD-2, mix for 15 min, transfer settled Amberlite XAD-2 to a 100 × 9 glass column, wash with 10 mL water, dry under a stream of nitrogen, elute with 25 mL MeOH:ethyl acetate 50:50. Evaporate eluate to dryness at 50° and take up residue in 2 mL 250 mM pH 4.8 acetate buffer. Add 50 μL Helix pomatia juice containing a minimum 40 U/mL β-glucuronidase and 20 U/mL arylsulfatase and incubate at 37° for 2 h. Add 4 drops 6 M HCl and 20 mL ethyl acetate, mix for 15 min, remove water layer, incubate ethyl acetate layer at 37° for 1 h, wash with two 3 mL portions of 10% $NaHCO_3$, wash with 3 mL water. Evaporate to dryness, dissolve in 70 mL MeCN:water 5:95, analyze a 53 mL aliquot. Bile. 3 mL Bile + 2 mL 250 mM pH 4.8 acetate buffer + 50 μL Helix pomatia juice containing a minimum 40 U/mL β-glucuronidase and 20 U/mL arylsulfatase, incubate at 37° for 2 h. Add 4 drops 6 M HCl and 20 mL ethyl acetate, mix for 15 min, remove water layer, incubate ethyl acetate layer at 37° for 1 h, wash with two 3 mL portions of 10% $NaHCO_3$, wash with 3 mL water. Evaporate to dryness, dissolve in 70 mL MeCN:water 5:95, analyze a 53 mL aliquot. Liver, kidney. Add 80 mL 100 mM pH 9.5 Tris buffer containing 20 mg subtilopeptidase A (11.6 U/mg) to 20 g minced sample, incubate at 60° for 3.5 h, filter over glass wool, add 6 g Amberlite XAD-2 to the filtrate, mix for 15 min, transfer settled Amberlite XAD-2 to a 100 × 9 glass column, wash with 10 mL water, dry under a stream of nitrogen, elute with five 10 mL portions of MeOH. Evaporate eluate to dryness and take up residue in 2 mL 250 mM pH 4.8 acetate buffer. Add 50 μL Helix pomatia juice containing a minimum 40 U/mL β-glucuronidase and 20 U/mL arylsulfatase and incubate at 37° for 2 h. Add 4 drops 6 M HCl and 20 mL ethyl acetate, mix for 15 min, remove water layer, incubate ethyl acetate layer at 37° for 1 h, wash with two 3 mL portions of 10% $NaHCO_3$, wash with 3 mL water. Evaporate to dryness, dissolve in 70 mL MeCN:water 5:95, analyze a 53 mL aliquot. Meat. Add 80 mL 100 mM pH 9.5 Tris buffer containing 20 mg subtilopeptidase A (11.6 U/mg) to 20 g minced sample, incubate at 60° for 3.5 h, filter over glass wool, add 6 g Amberlite XAD-2 to the filtrate, mix for 15 min, transfer settled Amberlite XAD-2 to a 100 × 9 glass column, wash with 10 mL water, dry under a stream of nitrogen, elute with five 10 mL portions of MeOH. Evaporate eluate to dryness and take up residue in 70 mL MeCN:water 5:95, analyze a 53 mL aliquot. Condition column A with 20 mL water then add 53 mL sample, flush column A with 10 mL water. Condition column B with 10 mL water. Elute column A onto column B with 20 mL water containing 250 μg/mL norgestrel and 5% MeCN. Switch column B into circuit with column C and elute with mobile phase. Recondition column A with MeOH:water 70:30.

HPLC VARIABLES

Column: A 10 × 10 immuno precolumn (with immunoglobulin G immobilized on cyanogen bromide-activated Sepharose 4B, prepared as J.Chromatogr. 1988, 452, 419-433); B 10 × 2 Chrompack reverse phase column; C Chromsep reverse phase guard column (Chrompack) + 100 × 3 5 μm Chromspher glass column

Mobile phase: MeCN:water 35:65

Flow rate: 0.4

Injection volume: 53000

Detector: UV 340

CHROMATOGRAM

Retention time: 7 (α), 6 (β)

OTHER SUBSTANCES

Simultaneous: nandrolone (at UV 247)

KEY WORDS

meat; liver; kidney; SPE; column-switching

REFERENCE

Haasnoot,W.; Schilt,R.; Hamers,A.R.; Huf,F.A.; Farjam,A.; Frei,R.W.; Brinkman,U.A. Determination of β-19-nortestosterone and its metabolite α-19-nortestosterone in biological samples at the sub parts per billion level by high-performance liquid chromatography with on-line immunoaffinity sample pretreatment, *J.Chromatogr.*, **1989**, *489*, 157–171.

SAMPLE

Matrix: formulations

Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM

Retention time: 10.8 (trenbolone acetate)
Limit of detection: 5 μg/mL

OTHER SUBSTANCES

Simultaneous: aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone, fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, benzyl benzoate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate

KEY WORDS

oils; tablets; suspensions

REFERENCE

Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE

Matrix: microsomal incubations
Sample preparation: Add 3 volumes of MeOH to the microsomal incubation, centrifuge at 10000 g for 30 min. Evaporate the supernatant to dryness under reduced pressure, dissolve the residue in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: RP-18 Hibar (Merck)
Mobile phase: MeCN:glacial acetic acid:water 19:1:80
Flow rate: 1
Detector: Radioactivity

CHROMATOGRAM

Retention time: 7.0

KEY WORDS

cow; liver; tritium labeled

REFERENCE

Evrard,P.; Maghuin-Rogister,G. In vitro metabolism of trenbolone: study of the formation of covalently bound residues, *Food Addit.Contam.*, **1987**, *5*, 59–65.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 5 μm Hypersil ODS

Mobile phase: MeOH:water 60:40
Injection volume: 250
Detector: UV

CHROMATOGRAM
Retention time: 3.8

OTHER SUBSTANCES
Simultaneous: diethylstilbestrol, nandrolone, zeranol, dienestrol, hexestrol, 17α-methyltestosterone, medroxyprogesterone

REFERENCE
Jansen,E.H.J.M.; Both-Miedema,R.; van den Berg,R.H. Application of optimization procedures for the separation of anabolic compounds by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 57–64.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 5 μL aliquot of a 10 μg/mL solution in MeOH.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:10 mM ammonium acetate buffer 45:55
Flow rate: 0.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 3.807

OTHER SUBSTANCES
Simultaneous: boldenone, epimethandienone, epitestosterone, fluoxymesterone, 6β-hydroxymethandienone, methandienone, norethindrone, oxymetholone (UV 280)

REFERENCE
Barrón,D.; Pascual,J.A.; Segura,J.; Barbosa,J. Prediction of LC retention of steroids using solvatochromic parameters, *Chromatographia*, **1995**, *41*, 573–580.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Bond Elut silica SPE cartridge with 5 mL EtOH and 5 mL cyclohexane. Homogenize 5 g tissue with 11 mL 40 mM pH 4.1 sodium acetate buffer. Add 500 μL 17 mg/mL β-glucuronidase (type H-5) in acetate buffer, mix. Incubate at 37° overnight. Mix with 5 mL 2 M aqueous tris(hydroxymethyl)aminomethane. Fill an Extrelut cartridge with 25% of an Extrelut 20 sachet, mix the remainder of the sachet with the sample and add it to the cartridge. Rinse the sample tube with 15 mL butan-2-ol:hexane 5:95 and add the rinse to the cartridge. Elute with 100 mL butan-2-ol:hexane 5:95. Extract the eluate with two 10 mL portions of MeCN. Evaporate the combined MeCN extracts to dryness after adding two 2 mL portions of heptane. Reconstitute the residue with 2 mL cyclohexane. Add to the SPE cartridge Wash the tube with two 2 mL and two 1 mL portions of cyclohexane. Add to the SPE cartridge. Wash with 2 mL cyclohexane. Elute with 5 mL acetone:cyclohexane 25:75. Evaporate the eluate and redissolve the residue in 400 μL MeOH and 3.6 mL 85° water. Cool to room temperature and add to an immunoaffinity column (Randox Laboratories, UK). Complete the transfer with three 1 mL portions of hot water, cooled before adding to the column. Wash with 2 mL 1 mM pH 10 carbonate buffer, elute with 4 mL MeOH:water 70:30. Dilute the eluate with 12 mL water. Inject a 4 mL aliquot onto column A and then backflush the contents of column A onto column B with mobile phase. Monitor the effluent from column B.

HPLC VARIABLES
Column: A Chromspher (type R2) (Chrompack); B 200 × 3 5 μm Chromspher C18 (Chrompack)
Mobile phase: MeCN:water 35:65
Flow rate: 0.5

Injection volume: 4000
Detector: UV 340

CHROMATOGRAM
Retention time: 8 (β epimer), 9 (α epimer)
Limit of detection: 100 pg/g

OTHER SUBSTANCES
Extracted: nandrolone

KEY WORDS
pig; cow; liver; corned beef; SPE; column-switching

REFERENCE
Stubbings,G.W.; Cooper,A.D.; Shepherd,M.J.; Croucher,M.; Airs,D.; Farrington,W.H.H.H.; Shearer,G. Determination of 19-nortestosterone and trenbolone in animal tissues by high-performance liquid chromatography with immunoaffinity clean-up, *Food Addit.Contam.*, **1998**, *15*, 293–301.

SAMPLE
Matrix: tissue
Sample preparation: Dry pack 60 × 8 mm glass columns with 250 mg Carbopack B (200-400 mesh) and 60 × 4 mm glass columns with 50 mg Amberlite CG-400 I (100-200 mesh). Wash Carbopack column with 5 mL MeOH, 15 mL dichloromethane:MeOH 70:30, and MeOH:water 85:15. Wash Amberlite column with 3 mL 0.5 M NaOH, 8 mL dichloromethane:MeOH 70:30, 1 mL water, and 3 mL 1 M HCl. Repeat this cycle 4 times. Finally pass through 20 mL 50 mM NaOH then 1 mL water. Keep column in water. (Process converts Amberlite to OH form.) Homogenize 1 g of tissue in 5 mL MeOH, sonicate 5 min, centrifuge at 6000 rpm for 10 min. Add another 5 mL MeOH to pellet and repeat. Combine supernatants, make up to 6.8 mL with MeOH, add 1.2 mL water. Pass through Carbopack column, wash column with 2 mL MeOH: water 85:15 then 2 mL MeOH, elute column with 8 mL dichloromethane:MeOH 70:30. Pass eluate onto Amberlite column, add 1 mL MeOH to column, collect all eluates from column, evaporate to dryness under nitrogen at 40°, take up in 200 μL MeOH:water 50:50, add 25 μL 10 μg/mL p-chlorophenol, inject 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-18
Column: 250 × 4.6 5 μm Supelco C18
Mobile phase: Gradient. MeCN:water from 40:60 to 65:35 in 30 min
Flow rate: 1.2
Injection volume: 50
Detector: UV 242

CHROMATOGRAM
Retention time: 24
Internal standard: p-chlorophenol (7)
Limit of detection: 1 ng/g

OTHER SUBSTANCES
Simultaneous: testosterone, progesterone

KEY WORDS
muscle; liver; chicken; ox; SPE

REFERENCE
Laganà,A.; Marino,A. General and selective isolation procedure for high-performance liquid chromatographic determination of anabolic steroids in tissues, *J.Chromatogr.*, **1991**, *588*, 89–98.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Waring blender) tissue at full speed for 2 min, lyophilize, grind. Extract with supercritical carbon dioxide at 60° at 400 atmospheres with a 20 cm × 21

μm restrictor for 1 h, collect the extract in 1 mL MeOH cooled to 5°. Evaporate the MeOH to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeCN:MeOH:20 mM ammonium formate 15:15:70, inject an aliquot. Alternatively, vortex 5 g ground tissue with 10 mL 40 mM sodium acetate, adjust pH to 4.2-4.7 with glacial acetic acid, add 100 μL β-glucuronidase (Sigma), heat at 37° for 8 h, add 20 mL MeCN, vortex for 30 s, centrifuge at 5000 rpm for 20 min. Remove a 30 mL aliquot of the supernatant and add it to 8 mL hexane and 2 mL dichloromethane, rotate for 3 min, centrifuge at 2000 rpm for 2 min. Remove a 15 mL aliquot of the middle layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, inject an aliquot.

HPLC VARIABLES

Column: 50 × 4.6 5 μm Supelcosil
Mobile phase: Gradient. MeCN:MeOH:20 mM ammonium formate from 2.5:2.5:95 to 47.5:47.5:5 over 19 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245 or MS, Sciex TAGA 6000E tandem triple quadrupole, APCI

CHROMATOGRAM

Retention time: 9.8
Limit of detection: 100 ppb

OTHER SUBSTANCES

Extracted: dexamethasone, diethylstilbestrol, medroxyprogesterone, melengestrol acetate, triamcinolone acetonide, zeranol

KEY WORDS

cow; muscle; liver; SFE

REFERENCE

Huopalahti,R.P.; Henion,J.D. Application of supercritical fluid extraction and high performance liquid chromatography/mass spectrometry for the determination of some anabolic agents directly from bovine tissue samples, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 69–87.

SAMPLE

Matrix: urine
Sample preparation: 10 mL Urine + glucuronidase/sulfatase (Helix pomatia), incubate at 37° for 1 h, extract twice with 5 mL diethyl ether, add 225 μL water and evaporate ether under nitrogen, add 400 μL MeOH, inject a 250 μL aliquot of this mixture.

HPLC VARIABLES

Guard column: 75 × 2.1 Corasil C18
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Flow rate: 2
Injection volume: 250
Detector: UV 240

CHROMATOGRAM

Retention time: 4.5
Limit of detection: about 6 ng/mL

OTHER SUBSTANCES

Simultaneous: 17α-methyltestosterone, zeranol, trans-diethylstilbestrol, medroxyprogesterone, nandrolone

KEY WORDS

cow

REFERENCE
Jansen,E.H.; Both-Miedema,R.; van Blitterswijk,H.; Stephany,R.W. Separation and purification of several anabolics present in bovine urine by isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *299*, 450–455.

SAMPLE
Matrix: urine
Sample preparation: Hydrolyze 1 mL urine with glucuronidase/sulfatase (from Helix pomatia, IBF; purified by gel filtration on Sephadex G-25M) at 37° for 2 h, cool, extract with ether. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 250 μL mobile phase, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil silica
Mobile phase: Isooctane:EtOH 97:3 for 8 min then 60:40 for 2 min to clean column
Flow rate: 2
Injection volume: 200
Detector: UV 350

CHROMATOGRAM
Retention time: 2.25 (trenbolone acetate), 6.2 (17β-trenbolone), 7.0 (17α-trenbolone)
Limit of detection: 1-2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, triendione

KEY WORDS
cow; normal phase

REFERENCE
Jansen,E.H.J.M.; Zoontjes,P.W.; van Blitterswijk,H.; Both-Miedema,R.; Stephany,R.W. Fast high-performance liquid chromatographic screening method for the presence of trenbolone and its major metabolite in urine of slaughter cattle, *J.Chromatogr.*, **1985**, *319*, 436–439.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH of 1 mL urine to 5,2 with 500 mM acetic acid, add 375 μL purified enzyme, heat at 37° for 1 h, cool, add to an immunosorbent column (preparation details in paper), wash with two 1 mL portions of water, wash with 5 mL water, elute with 5 mL EtOH:water 40:60. Evaporate the eluate to 800 μL under a stream of nitrogen, make up to 1 mL with water, extract with 6 mL diethyl ether. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 180 μL aliquot. (Enzyme was suc d'Helix pomatia (Industrie Biologique Francaise) purified by gel filtration on Pharmacia PD-10.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm LiChrosorb diol
Mobile phase: Isooctane:EtOH 95:5
Flow rate: 2
Injection volume: 180
Detector: UV 350 (with confirmation by TLC)

CHROMATOGRAM
Retention time: 6.1 (17β- and 17α-trenbolone)
Limit of detection: 2 ng/mL

KEY WORDS
cow

REFERENCE
van Ginkel,L.A.; van Blitterswijk,H.; Zoontjes,P.W.; van den Bosch,D.; Stephany,R.W. Assay for trenbolone and its metabolite 17 α-trenbolone in bovine urine based on immunoaffinity chromatographic clean-up and off-line high-performance liquid chromatography-thin-layer chromatography, *J.Chromatogr.*, **1988**, *445*, 385–392.

SAMPLE
Matrix: urine
Sample preparation: Add urine to an Amberlite XAD-2 column, elute with MeOH, add eluate to a column of neutral alumina (activity 1, Merck), elute with EtOH:water 96:4 (unconjugated), elute with water (sulfates), elute with 40 mM pH 6.0 citrate-phosphate buffer (glucuronides). Hydrolyze conjugates with β-glucuronidase/sulfatase (Helix pomatia, Serva), extract with ethyl acetate, evaporate the organic layer under reduced pressure, reconstitute the residue with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Zorbax ODS
Mobile phase: Gradient. A was MeOH:water 10:90. B was MeCN. A:B from 85:15 to 63:37 over 10 min (concave gradient, Waters curve 8), maintain at 63:37 for 10 min, to 0:100 over 5 min.
Flow rate: 1
Detector: UV 340 or radioactivity

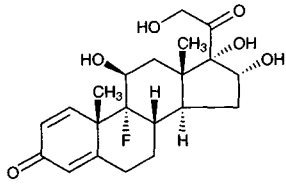
CHROMATOGRAM
Retention time: 33 (17β-trenbolone), 35 (17α-trenbolone)

OTHER SUBSTANCES
Extracted: metabolites, trendione

KEY WORDS
tritium labeled; SPE

REFERENCE
Spranger,B.; Metzler,M. Disposition of 17β-trenbolone in humans, *J.Chromatogr.*, **1991**, *564*, 485–492.

Triamcinolone



Molecular formula: $C_{21}H_{27}FO_6$
Molecular weight: 394.44
CAS Registry No.: 124-94-7, 76-25-5 (acetonide), 1997-15-5 (acetonide disodium phosphate), 31002-79-6 (benetonide), 5611-51-8 (hexacetonide), 67-78-7 (diacetate), 4989-94-0 (furetonide), 989-96-8 (21-(dihydrogen phosphate)
Merck Index: 9727
Lednicer No.: 1 201; 2 302

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL MeOH and 50 μL 1 μg/mL fluocortolone in MeOH to 1 mL plasma. Add 500 μL 100 mM NaOH and 2 mL dichloromethane, shake for 10 min, centrifuge at 2500 g for 10 min, evaporate a 1.9 mL aliquot of the supernatant under a stream of nitrogen at 45°. Reconstitute the residue in 50 μL MeOH, inject 17 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4 5 μm LiChrospher RP 18
Column: 250 × 4 5 μm Lichrospher RP 18
Mobile phase: MeOH:THF:water 110:2.5:100
Flow rate: 1

Injection volume: 17
Detector: UV 252

CHROMATOGRAM
Internal standard: fluocortolone
Limit of quantitation: 600 pg/mL

OTHER SUBSTANCES
Extracted: hydrocortisone

KEY WORDS
plasma

REFERENCE
Doppenschmitt,S.A.; Scheidel,B.; Harrison,F.; Surmann,J.P. Simultaneous determination of triamcinolone acetonide and hydrocortisone in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *682*, 79–88.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 2.5 mg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:water 36:64
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 6.4 (triamcinolone acetonide)

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Cavina,G.; Alimenti,R.; Gallinella,B.; Valvo,L. The identification of related substances in triamcinolone acetonide by means of high-performance liquid chromatography with diode array detector and mass spectrometry, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 685–692.

SAMPLE
Matrix: formulations
Sample preparation: Dermatological patch (2 cm × 2 cm) + 2 mL hexane, shake mechanically for 10 min, add 8 mL mobile phase, mix thoroughly, centrifuge at 2500 rpm for 10 min, remove 1 mL of lower phase, inject a 10 μL aliquot of this solution.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb C8
Mobile phase: MeOH:water 70:30
Flow rate: 0.5
Injection volume: 10
Detector: UV 240

KEY WORDS
for triamcinolone acetonide; dermatological patches; stability-indicating

REFERENCE
Edwardson,P.A.D.; Gardner,R.S. Problems associated with the extraction and analysis of triamcinolone acetonide in dermatological patches, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 935–938.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out amount containing 10 mg triamcinolone acetonide, make up to 50 mL with mobile phase. Remove a 2 mL aliquot and dilute it to 10 mL with mobile phase, filter, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water:96% acetic acid 55:44:1, pH 3.0
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.02 (triamcinolone acetonide)

OTHER SUBSTANCES
Simultaneous: salicylic acid

KEY WORDS
topical solution

REFERENCE
Kedor-Hackmann,E.R.M.; Gianotto,E.A.S.; Santoro,M.I.R.M. Determination of triamcinolone acetonide and salicylic acid in pharmaceutical formulations by high performance liquid chromatography, *Pharmazie*, **1996**, *51*, 63–63.

SAMPLE
Matrix: formulations, solutions
Sample preparation: Ointment. 1 g Ointment + 5 mL MeOH + 5 mL water + 800 μL 1 mg/mL hydrocortisone in EtOH, stir until a clear solution forms, make up to 25 mL with water, inject a 20 μL aliquot. Solutions. 8 mL Solution + 800 μL 1 mg/mL hydrocortisone in EtOH + 5 mL MeOH, make up to 25 mL with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeCN:200 mM KH_2PO_4 32:68, pH 4.2
Flow rate: 3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8 (triamcinolone acetonide)
Internal standard: hydrocortisone (4)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
ointment; stability-indicating

REFERENCE
Das Gupta,V. Stability of triamcinolone acetonide solutions as determined by high-performance liquid chromatography, *J.Pharm.Sci.*, **1983**, *72*, 1453–1456.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: μBondapak ODS

Mobile phase: MeCN:water 30:70
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 12 (triamcinolone acetonide)
Internal standard: fluoxymesterone (10)

REFERENCE
Kirschbaum,J. High-pressure liquid chromatography of triamcinolone acetonide: effect of different octadecylsilane columns on mobility, *J.Pharm.Sci.*, **1980**, *69*, 481–482.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Waring blender) tissue at full speed for 2 min, lyophilize, grind. Extract with supercritical carbon dioxide at 60° at 400 atmospheres with a 20 cm × 21 μm restrictor for 1 h, collect the extract in 1 mL MeOH cooled to 5°. Evaporate the MeOH to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeCN:MeOH:20 mM ammonium formate 15:15:70, inject an aliquot. Alternatively, vortex 5 g ground tissue with 10 mL 40 mM sodium acetate, adjust pH to 4.2-4.7 with glacial acetic acid, add 100 μL β-glucuronidase (Sigma), heat at 37° for 8 h, add 20 mL MeCN, vortex for 30 s, centrifuge at 5000 rpm for 20 min. Remove a 30 mL aliquot of the supernatant and add it to 8 mL hexane and 2 mL dichloromethane, rotate for 3 min, centrifuge at 2000 rpm for 2 min. Remove a 15 mL aliquot of the middle layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, inject an aliquot.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil
Mobile phase: Gradient. MeCN:MeOH:20 mM ammonium formate from 2.5:2.5:95 to 47.5:47.5:5 over 19 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245 or MS, Sciex TAGA 6000E tandem triple quadrupole, APCI

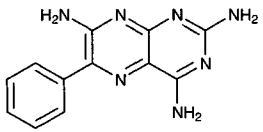
CHROMATOGRAM
Retention time: 10 (triamcinolone acetonide)
Limit of detection: 100 ppb

OTHER SUBSTANCES
Extracted: dexamethasone, diethylstilbestrol, medroxyprogesterone, melengestrol acetate, trenbolone, zeranol

KEY WORDS
cow; muscle; liver; SFE

REFERENCE
Huopalahti,R.P.; Henion,J.D. Application of supercritical fluid extraction and high performance liquid chromatography/mass spectrometry for the determination of some anabolic agents directly from bovine tissue samples, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 69–87.

Triamterene



Molecular formula: $C_{12}H_{11}N_7$
Molecular weight: 253.27
CAS Registry No.: 396-01-0
Merck Index: 9731
Lednicer No.: 1 427

SAMPLE

Matrix: blood

Sample preparation: Add 1.5 mL MeCN to 500 μL serum, centrifuge, evaporate the supernatant to dryness, redissolve the residue in 200 μL water. Inject onto column A, wash with MeCN:water 10:90 or MeOH:water 20:80 for 20 min, backflush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 25 × 4 25 μm pore diameter 6 nm LiChrospher RP-18 ADS (Merck); B 125 × 4 5 μm endcapped LiChroCART HPLC-cartridge RP-18 (Merck)

Mobile phase: MeOH:20 mM pH 4 phosphate buffer 38:62

Column temperature: 40

Flow rate: 1

Injection volume: 200

Detector: UV 245, F ex 270 em 389

CHROMATOGRAM

Retention time: 2.7

OTHER SUBSTANCES

Extracted: trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Oertel,R.; Richter,K.; Gramatté,T.; Kirch,W. Determination of drugs in biological fluids by high-performance liquid chromatography with on-line sample processing, *J.Chromatogr.A*, **1998**, *797*, 203–209.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 215.8

CHROMATOGRAM

Retention time: 8.705

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations, urine
Sample preparation: Tablets. Pulverize tablets, add MeOH, shake for 30 min, sonicate for 5 min, filter (Albet 242 paper), wash solid with MeOH, make up filtrate to 50 mL with MeOH, inject a 20 μL aliquot. Urine. Adjust pH of 2 mL urine to 10.0 with 2 M KOH, add 1.5 mg NaCl, add 4 mL ethyl acetate, shake for 10 min, centrifuge at 2500 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 2 mL mobile phase, sonicate, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 30:70 containing 5 mM KH_2PO_4/K_2HPO_4, pH adjusted to 5.5
Flow rate: 1
Injection volume: 20
Detector: E, EG&G Princeton Applied Research PAR Model 400, glassy carbon working electrode +1300 mV, Ag/AgCl reference electrode (At the end of each day clean electrode with mobile phase of MeOH at 1.5 mL/min, -800 mV for 2 min then +1600 mV for 5 min.)

CHROMATOGRAM
Retention time: 5.01
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: furosemide

KEY WORDS
tablets; pharmacokinetics

REFERENCE
Barroso,M.B.; Alonso,R.M.; Jiménez,R.M. Simultaneous determination of the diuretics triamterene and furosemide in pharmaceutical formulations and urine by HPLC-EC, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 231–246.

SAMPLE
Matrix: urine
Sample preparation: Inject 5 μL urine onto column A and elute to waste with mobile phase A, after 1 min backflush the contents of column A onto column B with mobile phase B. Monitor the effluent from column B.

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 125 × 4 5 μm LiChrospher 100 RP 18
Mobile phase: A 50 mM pH 3 phosphate buffer; B MeCN:50 mM pH 3 phosphate buffer 60:40 (Prepare buffer as follows. Dissolve 3.45 g NaH_2PO_4 monohydrate in 500 mL water containing 750 μL propylamine hydrochloride, adjust to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 254, F ex 365 em 440

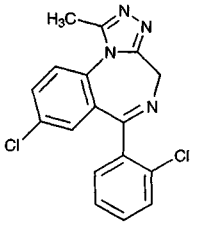
CHROMATOGRAM
Retention time: 5.5
Limit of detection: 10 pg/mL

OTHER SUBSTANCES
Extracted: amiloride, bumetanide, furosemide

KEY WORDS
column-switching

REFERENCE
Campins-Falcó,P.; Herráez-Hernández,R.; Pastor-Navarro,M.D. Analysis of diuretics in urine by column-switching chromatography and fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 1867–1885.

Triazolam



Molecular formula: $C_{17}H_{12}Cl_2N_4$
Molecular weight: 343.21
CAS Registry No.: 28911-01-5
Merck Index: 9734
Lednicer No.: 1 368

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum + 20 µL 20 µg/mL IS + 200 µL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100 µL mobile phase, inject a 20 µL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES

Column: 100 × 4.6 2 µm TSK gel Super-ODS (A) or 100 × 4.6 5 µm Hypersil ODS-C18 (B)
Mobile phase: MeCN:5 mM pH 6 NaH_2PO_4 45:55
Flow rate: 0.65
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 13.7 (A), 43.5 (B)
Internal standard: diazepam (29.8 (A), 77.5 (B))
Limit of quantitation: 5 ng/mL (A)

OTHER SUBSTANCES

Extracted: bromazepam, chlordiazepoxide, clonazepam, estazolam, etizolam, flutazolam, haloxazolam, lorazepam, nitrazepam, oxazolam
Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione
Interfering: alprazolam

KEY WORDS

serum

REFERENCE

Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-µm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Serum or plasma + 100 µL 1 µg/mL IS in water + 0.5 mL water, vortex, extract with 10 mL toluene:isoamyl alcohol 99:1 for 10 min on a rotator, centrifuge for 5 min. Remove upper organic layer, evaporate under a stream of nitrogen at 37°, take up in 150 µL mobile phase, vortex for 2 min, add 0.5 mL hexane, vortex briefly, centrifuge for 5 min, discard upper hexane layer, inject a 100 µL aliquot of the lower layer.

HPLC VARIABLES

Column: 250 × 4 Bio-Sil ODS-10 (Bio-Rad)
Mobile phase: MeCN:pH 4.5 50 mM phosphate buffer 30:70 (Buffer was 6.9 g KH_2PO_4 in 1 L adjusted to pH 4.5 with orthophosphoric acid.)
Column temperature: 45
Flow rate: 2.5
Injection volume: 100
Detector: UV 202

CHROMATOGRAM
Retention time: 8.4
Internal standard: U-31485 (6.9)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: desipramine, protriptyline
Noninterfering: N-acetylprocainamide, amitriptyline, caffeine, chlordiazepoxide, chlorpromazine, diazepam, flurazepam, lorazepam, oxazepam, prazepam, procainamide, propranolol, thioridazine
Interfering: alprazolam, imipramine, nortriptyline

KEY WORDS
plasma; serum

REFERENCE
McCormick,S.R.; Nielsen,J.; Jatlow,P. Quantification of alprazolam in serum or plasma by liquid chromatography, *Clin.Chem.*, **1984**, *30*, 1652–1655.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 2 mL water + 2 mL 100 mM NaOH, mix gently, add 8 mL diethyl ether, shake for 15 min, centrifuge at 2500 rpm for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, vortex for 30 s, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 50 × 4.6 Shim-pack FLC-C8 (Shimadzu)
Mobile phase: MeOH:buffer 53:47 (Buffer was 5 mM Na_2HPO_4 adjusted to pH 6.0 with phosphoric acid.)
Flow rate: 0.6
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 3.7

OTHER SUBSTANCES
Extracted: diazepam, nordiazepam, clorazepate, temazepam, oxazepam
Simultaneous: sulpride, bromazepam, nitrazepam, flunitrazepam
Noninterfering: haloperidol, trihexyphenidyl
Interfering: estazolam

KEY WORDS
serum

REFERENCE
Tada,K.; Moroji,T.; Sekiguchi,R.; Motomura,H.; Noguchi,T. Liquid-chromatographic assay of diazepam and its major metabolites in serum, and application to pharmacokinetic study of high doses of diazepam in schizophrenics, *Clin.Chem.*, **1985**, *31*, 1712–1715.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 4 μg/mL triazolam in methanol + 0.5 mL pH 9.12 saturated solution of sodium borate + 4.0 mL ethyl acetate:heptane 85:15, shake 10 min, centrifuge at 220 g for 10 min. Remove organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm C18 (IBM)
Mobile phase: MeCN:50 mM pH 6 potassium phosphate 30:70

Flow rate: 1.5
Detector: UV 214

CHROMATOGRAM
Retention time: 13.2
Internal standard: triazolam

OTHER SUBSTANCES
Extracted: alprazolam
Simultaneous: nitrazepam

KEY WORDS
plasma; between injections wash column with 10 mL MeCN:water 70:30 then 10 mL mobile phase; triazolam is IS

REFERENCE
Miller,R.L.; DeVane,C.L. Alprazolam, α-hydroxy- and 4-hydroxyalprazolam analysis in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *430*, 180–186.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 20 μL 2.5 μg/mL norprazepam in MeOH + 50 μL buffer + 6 mL diethyl ether:dichloromethane 2:1, agitate, centrifuge. Remove the organic phase and evaporate to dryness under vacuum at 45°, dissolve the residue in 50 μL MeOH, inject a 20 μL aliquot. (Prepare buffer as follows. Solution A was 6.18 g boric acid + 7.46 g KCl in 100 mL water. Solution B was 10.6 g sodium carbonate in 100 mL water. Mix 63 mL solution A and 37 mL solution B and adjust pH to 9.5.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nova Pak C18
Mobile phase: MeCN:MeOH:buffer 23:13:64 (Buffer was 94 mL 200 mM NaH_2PO_4 + 6 mL 200 mM Na_2HPO_4, adjusted to pH 5.0 with 100 mM HCl.)
Flow rate: 1.3
Injection volume: 20
Detector: UV 242

CHROMATOGRAM
Retention time: 14.9
Internal standard: norprazepam (18.6)
Limit of quantitation: 30 ng/mL

OTHER SUBSTANCES
Simultaneous: alprazolam, bromazepam, chlordiazepoxide, clonazepam, diazepam, estazolam, flumazenil, flunitrazepam, loflazepate, lorazepam, nitrazepam, norflunitrazepam, oxazepam
Noninterfering: acepromazine, aceprometazine, amylobarbital, aprobarbital, barbital, brallobarbital, butalbital, caffeine, carbamazepine, chlorpromazine, cyclobarbital, ethosuximide, heptabarbital, hexobarbital, loprazolam, medazepam, midazolam, pentobarbital, phenobarbital, phenytoin, prazepam, secobarbital, theophylline, thiopental, vinylbarbital
Interfering: tofizopam, clobazam

KEY WORDS
plasma

REFERENCE
Boukhabza,A.; Lugnier,A.A.; Kintz,P.; Mangin,P. Simultaneous HPLC analysis of the hypnotic benzodiazepines nitrazepam, estazolam, flunitrazepam, and triazolam in plasma, *J.Anal.Toxicol.*, **1991**, *15*, 319–322.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 25 μL 1 μg/mL triazolam in toluene + 75 μL 0.1% ammonium hydroxide, vortex 30 s, add 5 mL methylene chloride + 5 mL toluene, shake 15 min,

centrifuge at 177 g for 10 min. Remove aqueous layer and freeze residual aqueous layer in dry ice-acetone for 30 s. Decant organic layer, dry under nitrogen at 50°, vortex residue with 200 μL mobile phase, inject a 125 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm C18 (Supelco)
Mobile phase: MeOH:buffer 40:60 (Buffer was 1 mM phosphate and 3 mM hexyltriethylammonium phosphate in water at pH 7.4.)
Column temperature: 35
Flow rate: 2
Injection volume: 125
Detector: UV 221

CHROMATOGRAM
Retention time: 30
Internal standard: triazolam
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: alprazolam

KEY WORDS
serum; triazolam is IS

REFERENCE
Schmith,V.D.; Cox,S.R.; Zemaitis,M.A.; Kroboth,P.D. New high-performance liquid chromatographic method for the determination of alprazolam and its metabolites in serum: instability of 4-hydroxyalprazolam, *J.Chromatogr.*, **1991**, *568*, 253–260.

SAMPLE
Matrix: blood
Sample preparation: 3 mL Plasma + 30 μL 10 μg/mL triazolam in water, mix 1 min, allow to stand for 15 min at room temperature, add to 3 mL Extrelut SPE cartridge and allow to soak in for 10 min, elute with 20 mL dichloromethane. Evaporate eluant at 30° under reduced pressure, take up residue in 1 mL MeCN:water 5:95, stand for 15 min, centrifuge at 14000 g for 2 min, remove supernatant. Inject a 250 μL aliquot of the supernatant onto column A with mobile phase A and elute to waste, after 7 min forward flush the contents of column A onto column B with mobile phase B, after 0.47 min remove column A from circuit and elute column B with mobile phase B, monitor the effluent from column B. When not in use flush column A with mobile phase A. Between injections clean column A with two injections of 250 μL MeCN.

HPLC VARIABLES
Column: A 30 × 2.1 10 μm MPLC cartridge PRP-1 (Kontron); B 100 × 4.6 MPLC cartridge 5 μm RP-8 Spheri-5 (Kontron)
Mobile phase: A 1 L water + 20 mL MeCN + 50 μL phosphoric acid (pH 3.2); B MeCN:buffer 40:60 (Buffer was 1 L water + 20 mL MeCN + 50 μL phosphoric acid (pH 3.2).)
Flow rate: A 0.3; B 1
Injection volume: 250
Detector: UV 230

CHROMATOGRAM
Retention time: 7.0
Internal standard: triazolam

OTHER SUBSTANCES
Extracted: alprazolam
Simultaneous: bromazepam, oxazepam, lorazepam, diazepam
Interfering: clobazam

KEY WORDS
plasma; SPE; column-switching; triazolam is IS

REFERENCE
Rieck,W.; Platt,D. High-performance liquid chromatographic method for the determination of alprazolam in plasma using the column-switching technique, *J.Chromatogr.*, **1992**, *578*, 259–263.

SAMPLE
Matrix: blood
Sample preparation: Inject 100-200 μL plasma onto column A with mobile phase A and elute to waste, after 5 min backflush the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Wash column A with MeCN:water 60:40 at 1 mL/min for 6 min then re-equilibrate with pH 7.5 buffer for 10 min.

HPLC VARIABLES
Column: A 45 × 4 12 μm TSK-gel G 3 PW (Tosohass); B 75 × 4.6 Ultrasphere ODS C18 3 μm
Mobile phase: A 50 mM pH 7.5 phosphate buffer; B Gradient. A was MeCN. B was 65 mM KH_2PO_4 + 1% diethylamine adjusted to pH 5.4 with phosphoric acid. A:B 22:78 for 5 min, to 25:75 over 10 min, to 60:40 over 15 min.
Flow rate: 1
Injection volume: 100-200
Detector: UV 230

CHROMATOGRAM
Retention time: 23.3

OTHER SUBSTANCES
Extracted: alprazolam, bromazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, clotiazepam, desmethylclobazam, desmethyldiazepam, diazepam, estazolam, loflazepate, lorazepam, medazepam, nitrazepam, oxazepam, prazepam, temazepam, tetrazepam, tofisopam
Noninterfering: carbamazepine, phenytoin, ethosuximide, phenobarbital, primidone, valproic acid
Interfering: flunitrazepam

KEY WORDS
plasma; column-switching

REFERENCE
Lacroix,C.; Wojciechowski,F.; Danger,P. Monitoring of benzodiazepines (clobazam, diazepam and their main active metabolites) in human plasma by column-switching high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *617*, 285–290.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 25 μL 0.5 μg/mL demoxepam in water + 100 μL 1 M pH 9.0 borate buffer, mix well, add 2 mL diethyl ether, vortex for 40 s, centrifuge at 1100 g for 5 min. Remove ether layer and evaporate it at 40° under nitrogen. Take up residue in 50 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 2 5μm Ultrasphere C18
Mobile phase: MeCN:MeOH:43 mM pH 2.4 sodium acetate buffer 8:45:47
Flow rate: 0.3
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: k' 6.05
Internal standard: demoxepam (4, k' 2.73)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, alprazolam

Simultaneous: chlorpromazine, clonazepam, diazepam, flurazepam, hexobarbital, oxazepam, phenobarbital, temazepam
Noninterfering: amphetamine, buspirone, chlordiazepoxide, cocaine, cocathylene, flumazenil, midazolam, norcocaine

KEY WORDS
serum; rat

REFERENCE
Jin,L.; Lau,C.E. Determination of alprazolam and its major metabolites in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats, *J.Chromatogr.B*, **1994**, *654*, 77–83.

SAMPLE
Matrix: blood
Sample preparation: Plasma. 1 mL Plasma + 20 μL 25 ng/mL diazepam in MeOH + 100 μL 5 M NaOH + 5 mL hexane:dichloromethane 50:50, shake mechanically for 10 min, centrifuge at 1300 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, sonicate, inject an aliquot. Urine. Adjust pH of 10 mL urine to 5 with acetic acid, add 2.5 mL 500 mM pH 5.0 acetate buffer, add 3000 U β-glucuronidase (type IX, Sigma), heat at 37° for 24 h, centrifuge at 1300 g for 10 min. 1 mL Supernatant + 20 μL 25 ng/mL diazepam in MeOH + 100 μL 5 M NaOH + 5 mL hexane: dichloromethane 50:50, shake mechanically for 10 min, centrifuge at 1300 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, sonicate, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS-HG-5
Mobile phase: Gradient. MeOH:50 mM pH 4.0 ammonium acetate from 50:50 to 100:0 over 15 min.
Injection volume: 200
Detector: MS, Hitachi M-1200H, APCI, positive ion, nebulizer 170°, desolvation 400°, needle-electrode 2700 V

CHROMATOGRAM
Retention time: 9
Internal standard: diazepam (12)
Limit of quantitation: 20 pg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; LC-MS

REFERENCE
Senda,N.; Kohta,K.; Takahashi,T.; Hizukuishi,K.; Mimura,T.; Fujita,T.; Nakayama,M. A highly sensitive method to quantify triazolam and its metabolites with liquid chromatography--mass spectrometry, *Biomed.Chromatogr.*, **1995**, *9*, 48–51.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 222

CHROMATOGRAM

Retention time: 3.88
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromet azine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 220.5

CHROMATOGRAM
Retention time: 17.353

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Cool microsomal incubation on ice, add 100 μL MeCN, add phenacetin, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 3.9 NovaPak C18
Mobile phase: MeCN:MeOH:50 mM phosphate buffer 22.5:10:67.5
Flow rate: 1.3
Detector: UV 220

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE
von Moltke,L.L.; Greenblatt,D.J.; Harmatz,J.S.; Duan,S.X.; Harrel,L.M.; Cotreau-Bibbo,M.M.; Pritchard,G.A.; Wright,C.E.; Shader,R.I. Triazolam biotransformation by human liver microsomes in vitro: Effects of metabolic inhibitors and clinical confirmation of a predicted interaction with ketoconazole, *J.Pharmacol.Exp.Ther.*, **1996**, *276*, 370–379.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.8 5 μm Nova-Pak C18
Mobile phase: MeCN:water:85% phosphoric acid:7.6 mM tetramethylammonium chloride 30:70:0.2:0.075, final apparent pH 6.7 (adjusted with 1 M NaOH)
Flow rate: 1
Injection volume: 15-30
Detector: UV 198 (nitrogen purged)

CHROMATOGRAM
Retention time: 11.3
Internal standard: triazolam

OTHER SUBSTANCES
Simultaneous: mezlocillin, phenobarbital, diazepam, oxazepam, clindamycin, clindamycin B
Noninterfering: cefoperazone, cefotaxime, cephalothin, ticarcillin

REFERENCE
La Follette,G.; Gambertoglio,J.; White,J.A.; Knuth,D.W.; Lin,E.T. Determination of clindamycin in plasma or serum by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1988**, *431*, 379–388.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 6.43 (A), 6.65 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: Heat 5 mL urine + 1 mL temazepam in MeOH with 1 mL β-glucuronidase at 37° for 2.5 h, cool, adjust to pH 8.5 with saturated Na_2CO_3, extract with 10 mL dichloromethane. Evaporate, take up the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Brownlee 5 μm RP-8
Mobile phase: MeCN:10 mM KH_2PO_4:n-nonylamine 450:550:0.6 adjusted to pH 3,2 with phosphoric acid
Flow rate: 1.6
Detector: UV 225

CHROMATOGRAM
Retention time: 8
Internal standard: temazepam (7)

OTHER SUBSTANCES
Extracted: metabolites
Interfering: alprazolam

REFERENCE
Fraser,A.D. Urinary screening for alprazolam, triazolam, and their metabolites with the EMIT d.a.u. benzodiazepine metabolite assay, *J.Anal.Toxicol.*, **1987**, *11*, 263–266.

SAMPLE
Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH, 10 mL dichloromethane:MeOH 9:1, 5 mL MeOH, and 10 mL water. Condition a Sep-Pak Silica SPE cartridge with 20 mL dichloromethane, 20 mL dichloromethane:MeOH 9:1, and 20 mL dichloromethane, then air dry. 10 mL Urine adjusted to pH 5 with acetic acid, add 2.5 mL 500 mM pH 5.0 acetate buffer, add 3000 U β-glucuronidase, incubate at 37° for 24 h, make alkaline with ammonia, centrifuge at 1200 g for 15 min, add the supernatant to the C18 SPE cartridge with 100 μL 5 μg/mL etizolam in MeOH, wash with 5 mL water, wash with 5 mL MeOH:water 20:80, wash with 2 mL water, elute with 7 mL dichloromethane:MeOH 9:1. Evaporate the eluate to dryness under vacuum, dissolve the residue in 5 mL dichloromethane:MeOH 99:1, add to the silica SPE cartridge, wash with 20 mL dichloromethane, wash with 25 mL dichloromethane:MeOH 99:1, elute with 20 mL dichloromethane:MeOH 9:1. Evaporate the eluate to dryness under vacuum, dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (MeOH for silica SPE cartridge was distilled and dried over 3 Å molecular sieve, then 0.1% water added just before use.)

HPLC VARIABLES
Column: 100 × 8 10 μm Radial-Pak C18
Mobile phase: MeOH:10 mM pH 8.0 phosphate buffer 65:35
Flow rate: 1
Injection volume: 20
Detector: UV 220

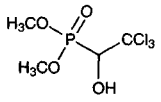
CHROMATOGRAM
Retention time: 14
Internal standard: etizolam (17)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
SPE

REFERENCE
Inoue,T.; Suzuki,S.-I. High-performance liquid chromatographic determination of triazolam and its metabolites in human urine, *J.Chromatogr.*, **1987**, *422*, 197–204.

Trichlorfon



Molecular formula: $C_4H_8Cl_3O_4P$
Molecular weight: 257.44
CAS Registry No.: 52-68-6
Merck Index: 9753

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 5 mL chloroform, vortex for 15 s, centrifuge at 0-5° at 800 g for 15 min, remove a 4.5 mL aliquot of the organic layer and add it to 3.5 mL 5 M HCl, vortex for 15 s, centrifuge at 0-5° at 800 g for 15 min, remove a 4 mL aliquot of the organic layer and add it to 200 mg anhydrous calcium sulfate (Drierite), vortex for 15 s, centrifuge at 0-5° at 800 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen (in an acid-washed tube), reconstitute the residue in 120 µL water, vortex, centrifuge at 0-5° at 11000 g for 5 min, inject a 100 µL aliquot of the supernatant. (Trichlorfon may degrade to dichlorvos during sample preparation unless whole blood is immediately acidified with phosphoric acid (J.Chromatogr. 1993,612, 336).)

HPLC VARIABLES
Guard column: 37-50 µm C18/Corasil
Column: 200 × 3.9 10 µm C18 (Waters)
Mobile phase: MeOH:THF:water 10:0.1:89.9 containing 1 mM octanesulfonic acid, pH 3.0
Flow rate: 2
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 10.8
Limit of detection: 1 µg/mL

KEY WORDS
plasma

REFERENCE
Unni,L.K.; Hannant,M.E.; Becker,R.E. High-performance liquid chromatographic method using ultraviolet detection for measuring metrifonate and dichlorvos levels in human plasma, *J.Chromatogr.*, **1992**, *573*, 99–103.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 5 mL chloroform, vortex for about 15 s, centrifuge at 0-5° at 800 g for 15 min. Remove the organic layer and add it to 3.5 mL 5 M HCl, vortex for 15 s, centrifuge at 0-5° at 800 g for 15 min. Remove a 4 mL aliquot of the organic layer and dry it over 200 mg anhydrous calcium sulfate (Drierite), vortex, centrifuge at 0-5° at 800 g for 5 min. Remove a 3.8 mL aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature in an acid-washed vial, reconstitute the residue in 120 µL water, vortex, centrifuge at 0-5° at 11000 g for 5 min, inject a 100 µL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 37-50 µm C18/Corasil
Column: 300 × 3.9 10 µm C18 (Waters)
Mobile phase: MeOH:THF:water 10:0.1:89.9 containing 1 mM sodium 1-octanesulfonate, pH adjusted to 3.0

Flow rate: 2
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 10.8
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Noninterfering: dichlorvos

KEY WORDS
plasma

REFERENCE
Unni,L.K.; Hannant,M.E.; Becker,R.E. High-performance liquid chromatographic method using ultraviolet detection for measuring metrifonate and dichlorvos levels in human plasma, *J.Chromatogr.*, **1992**, *573*, 99–103.

SAMPLE
Matrix: formulations
Sample preparation: 1 g Paste + 75 mL MeOH, sonicate for 15 min, shake for 1 min, sonicate for 5 min, shake for 30 s, cool to room temperature, make up to 100 mL with MeOH, centrifuge an aliquot at 2000 rpm for 10 min. Mix 2 mL supernatant with 2 mL 200 μg/mL methyl paraben in MeOH, make up to 25 mL with MeCN:water:phosphoric acid 20:80:1, filter (Millipore 0.6 μm polyvic), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 70 × 2.1 CO:PELL ODS
Column: 250 × 4.6 Partisil-5 ODS-3
Mobile phase: MeCN:buffer 20:80 (Buffer was 720 mL 1.38 g/L $NaH_2PO_4.H_2O$ + 80 mL 1.42 g/L Na_2HPO_4.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 200

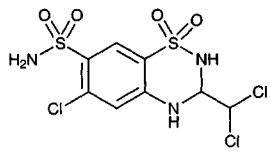
CHROMATOGRAM
Retention time: 6.69
Internal standard: methyl paraben (15.71)

OTHER SUBSTANCES
Simultaneous: oxfendazole

KEY WORDS
horse; paste

REFERENCE
Fleitman,J.; Neu,D.; Benjamin,E. Analysis of pharmaceutical dosage forms for oxfendazole: II. Simultaneous liquid chromatographic determination of oxfendazole and trichlorfon in equine paste, *J.Assoc.Off. Anal.Chem.*, **1986**, *69*, 24–28.

Trichlormethiazide



Molecular formula: $C_8H_8Cl_3N_3O_4S_2$
Molecular weight: 380.66
CAS Registry No.: 133-67-5
Merck Index: 9754
Lednicer No.: 1 359

SAMPLE

Matrix: blood
Sample preparation: 3 mL Plasma + 1 mL 10 mM NaOH + 1 mL 2 μg/mL bendroflumethiazide in 10 mM NaOH + 2 mL 10 mM HCl, mix, add 10 mL diethyl ether, shake gently on a platform shaker for 15 min, centrifuge at -10° at 2200 g for 15 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 70° for 45 min (these conditions are required to remove residual benzyl alcohol that is present as a preservative in the heparin), reconstitute the residue in 50 μL 10 mM NaOH, vortex for 25 s, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Isopropanol:water:acetic acid 17:82:1
Flow rate: 2
Injection volume: 20
Detector: UV 269

CHROMATOGRAM

Retention time: 3.7
Internal standard: bendroflumethiazide (14.4)
Limit of detection: 10 ng/mL

KEY WORDS

plasma; silanize glassware; pharmacokinetics

REFERENCE

Meyer,M.C.; Hwang,P.T.R. Determination of trichlormethiazide in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *223*, 466–472.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 225.2

CHROMATOGRAM
Retention time: 14.907

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetra-

cycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 500 mg solid sodium bicarbonate, mix, add 1 mL 10 mM NaOH, add 1 mL 2 μg/mL bendroflumethiazide in 10 mM NaOH, mix, add 10 mL diethyl ether, shake gently on a platform shaker for 15 min, centrifuge at -10° at 2200 g for 15 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 70° for 45 min (these conditions are required to remove residual benzyl alcohol that is present as a preservative in the heparin), reconstitute the residue in 100 μL MeOH, vortex for 25 s, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water:acetic acid 5:35:59:1
Flow rate: 2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 3.7
Internal standard: bendroflumethiazide (10.7)
Limit of detection: 50 ng/mL

KEY WORDS

silanize glassware; pharmacokinetics

REFERENCE

Meyer,M.C.; Hwang,P.T.R. Determination of trichlormethiazide in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *223*, 466–472.

SAMPLE

Matrix: urine

Sample preparation: 500 μL Urine + 1 mL 10 mM pH 7.0 phosphate buffer + 3 g NaCl + 9 mL ether, shake vigorously for 10 min, centrifuge at 2500 rpm for 10 min. Remove 7 mL of the organic layer and evaporate it to dryness using a centrifugal evaporator at 50°, reconstitute the residue in 100 μL 50 μg/mL methyl p-hydroxybenzoate in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Zorbax ODS
Mobile phase: MeCN:10 mM pH 7.0 phosphate buffer 20:80
Column temperature: 35
Flow rate: 0.8
Injection volume: 20
Detector: UV 274

CHROMATOGRAM

Internal standard: methyl p-hydroxybenzoate
Limit of detection: 2 μg/mL

KEY WORDS

pharmacokinetics

REFERENCE

Takahashi,H.; Watanabe,Y.; Shimamura,H.; Sugito,K. Effects of magnesium oxide on trichlormethiazide bioavailability, *J.Pharm.Sci.*, **1985**, *74*, 862–865.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 11.1 (A), 12.0 (B)

Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone, flumethiazide

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES

Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18

Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)

Flow rate: 1

Injection volume: 40

Detector: UV 230

CHROMATOGRAM

Retention time: 15.6

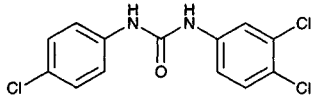
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene

KEY WORDS
column-switching; optimum detection wavelengths vary for each drug

REFERENCE
Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

Triclocarban



Molecular formula: $C_{13}H_9Cl3N_2O$
Molecular weight: 315.59
CAS Registry No.: 101-20-2
Merck Index: 9786

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

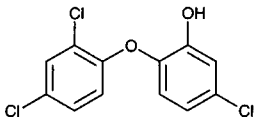
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 264.1

CHROMATOGRAM
Retention time: 25.573

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Triclosan



Molecular formula: $C_{12}H_{17}Cl_3O_2$
Molecular weight: 289.54
CAS Registry No.: 3380-34-5
Merck Index: 9790

SAMPLE

Matrix: formulations
Sample preparation: 2 g Dentifrice + 25 mL MeCN:water 60:40 + four 4 mm glass beads, vortex for 4 min, centrifuge at 10-20° at 13000 rpm for 15 min, repeat extraction twice. Combine the supernatants and make up to 100 mL with MeCN:water 60:40, vortex. Dilute 1:20, vortex, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: Nova-Pak C18 Guard-Pak
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:water 60:40
Flow rate: 1.5
Injection volume: 10
Detector: UV 280

CHROMATOGRAM

Retention time: 4.5

OTHER SUBSTANCES

Noninterfering: excipients

KEY WORDS

dentifrice; toothpaste

REFERENCE

Demkowicz,M.P.; Chauhan,V.; Stern,D.A.; Vasquez,F.G. Simultaneous determination of anions and triclosan in dentifrices by gradient ion chromatography and isocratic high-performance liquid chromatography interfaced with conductivity and ultraviolet detection, *J.Chromatogr.A*, **1994**, *671*, 351–357.

SAMPLE

Matrix: textiles
Sample preparation: Reflux 1 g fabric with 20 mL MeOH:acetic acid 90:10 for 30 min, filter (glass, 3G2), wash solid with 50 mL MeOH. Combine filtrate and washings and evaporate them to 5 mL under reduced pressure, add 10 mL 100 mM HCl, extract three times with 10 mL portions of diethyl ether. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 2 mL MeOH, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 TSK gel ODS-80TM
Mobile phase: MeCN:water:acetic acid 60:40:0.1
Column temperature: 37
Flow rate: 1
Detector: UV 240

CHROMATOGRAM

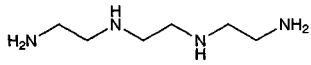
Retention time: 15

OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE
Kanetoshi,A.; Ogawa,H.; Katsura,E.; Kaneshima,H. Chlorination of Irgasan DP300 and formation of dioxins from its chlorinated derivatives, *J.Chromatogr.*, **1987**, *389*, 139–153.

Trientine



Molecular formula: $C_6H_{18}N_4$
Molecular weight: 146.24
CAS Registry No.: 112-24-3, 38260-01-4 (HCl)
Merck Index: 9796

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut SCX SPE cartridge with 5 mL water. 500 μL Plasma + 100 μL water + 1 mL MeCN, vortex briefly, centrifuge at 1000 g for 5 min. Add 1.2 mL of the supernatant to the SPE cartridge, wash with 3 mL water, wash with 2 mL 1 M KCl, wash with 3 mL 2 M KCl, elute with 1 mL 4 M KCl. Remove a 200 μL aliquot of the eluate and add it to 600 μL 100 mM pH 9.5 sodium phosphate buffer and 100 μL 0.15 mM trisodium EDTA in 100 mM pH 9.5 sodium phosphate buffer, mix, add 100 μL 10 mM fluorescamine in MeCN, vortex vigorously for 1 min, let stand for 20 min, add 50 μL 0.25 mM α-naphthylamine (Caution! α-Naphthylamine is a carcinogen!) in MeOH, inject a 20-50 μL aliquot.

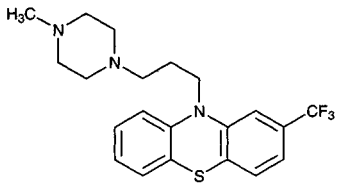
HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 5-CN
Mobile phase: MeCN:buffer 27:73 , pH adjusted to 6.0 with 2 M NaOH (Buffer was 140 mM ammonium chloride containing 48 mM sodium benzenesulfonate and 9.2 mM acetic acid.)
Column temperature: 40
Flow rate: 0.5
Injection volume: 20-50
Detector: F ex 380 em 485

CHROMATOGRAM
Retention time: 9.5
Internal standard: α-naphthylamine (13)
Limit of detection: 100 ng/mL

KEY WORDS
plasma; derivatization; SPE; rat; human; pharmacokinetics

REFERENCE
Miyazaki,K.; Kishino,S.; Kobayashi,M.; Arashima,S.; Matsumoto,S.; Arita,T. Determination of triethylenetetramine in plasma of patients by high-performance liquid chromatography, *Chem.Pharm.Bull.(Tokyo)*, **1990**, *38*, 1035–1038.

Trifluoperazine



Molecular formula: $C_{21}H_{24}F_3N_3S$
Molecular weight: 407.50
CAS Registry No.: 117-89-5, 440-17-5 (di HCl)
Merck Index: 9811

SAMPLE
Matrix: blood